Effects of oxygen on aerosol survival of radiation sensitive and resistant strains of *Escherichia coli* B

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SUMMARY

The aerosol survivals in air and nitrogen of radiation sensitive and resistant mutants of *Escherichia coli* B have been determined with logarithmic and resting phase bacteria. No consistent correlation was found between radiation sensitivity and aerosol sensitivity in the strains tested. Hence, the phenotypes Fil Her Exr, which determine sensitivity to radiation, do not influence aerosol survival, i.e. these known mechanisms which repair radiation-induced damage do not operate in aerosol stressed *E. coli*. In all cases the survival in air was less than that in nitrogen particularly so for *E. coli* B_{s-1}. The effect is explained in terms of a toxic action of oxygen. Comparison of survival of log and resting phase bacteria show that log phase cells are less aerosol stable than are resting phase cells. The ability to synthesize DNA in bacteria collected from the aerosol was less than in control unstressed bacteria, and this effect was independent of the presence of oxygen. Reduced ability to synthesize DNA could have been caused by reduced metabolic activity. It is shown that two different death mechanisms occur simultaneously in aerosols at low relative humidity. One mechanism is oxygen dependent and the other oxygen independent. The former was not through a decrease in metabolic activity, whereas the latter could be.

INTRODUCTION

Aerosol survival of micro-organisms has been reviewed in general terms by Anderson & Cox (1967). For bacteria there appears to be at least two different death mechanisms. There is a toxic action of oxygen (Ferry, Brown & Damon, 1958; Hess, 1965; Cox, 1966a, 1968b, 1970, 1971; Cox & Baldwin, 1966, 1967; Benbough, 1967, 1969; Webb, 1967, 1969) the effect of which occurs below 70 % relative humidity (RH) (Cox, 1966a, 1968b; Benbough, 1967, 1969), although Webb (1967, 1969) did not observe the effect above 40 % RH. Cox & Heckley (1972) have described a kinetic model which accounts for the toxic action of oxygen and indicates that the normally detected free radicals are not involved in oxygen toxicity. The other death mechanism is owing to dehydration and rehydration (Cox, 1965, 1966a, b, 1967, 1968a, b, 1969, 1970, 1971; Cox & Baldwin, 1966; Hatch &

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12

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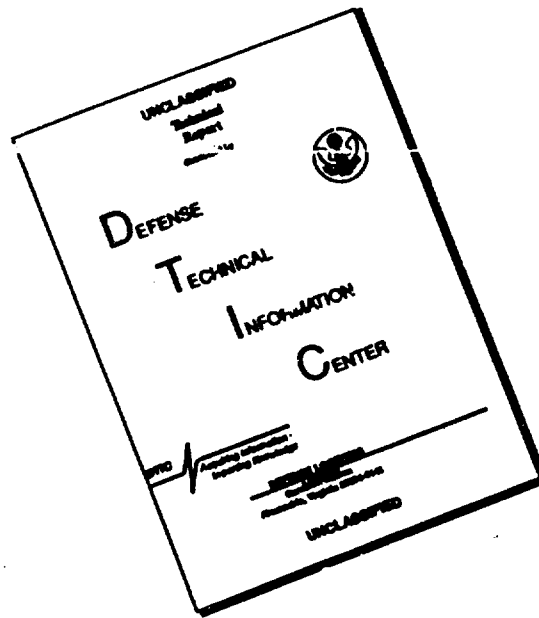
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Dimmick, 1965, 1966; Hatch & Warren, 1969; Hatch & Wolochow, 1969; Hatch, Wright & Bailey, 1970). However, Webb (1965, 1967, 1969) does not consider that rehydration is important with regard to aerosol survival. It is possible for the two death mechanisms to occur simultaneously, especially at low RH (Cox & Heckley, 1972).

Recently Webb (1969) has suggested that mechanisms exist which repair damage caused by dehydration in the aerosol and implies that these mechanisms are those involved in repair of UV damaged DNA. However, Webb (1969) only considered excision repair. The present paper investigates excision repair, filament formation and the mechanism involved in repair of damage caused by X-rays. The approach was to measure the aerosol survival in air and nitrogen of five radiation resistant and sensitive mutants derived from *Escherichia coli* B. Also, the net DNA synthesizing ability, and the metabolic activity, of some of these mutants was measured after the bacteria were stressed in the aerosol and collected.

MATERIALS AND METHODS

Organisms

The *Escherichia coli* strains used in this study are listed below.

Strain	Phenotype	Derived from	Source	UV Sensitivity
B/r (Hill)	Fil⁻Her⁺Exr⁺	B	Hill	Resistant
B/r (Witkin)	Fil⁻Her⁻Exr⁺	B	Witkin	Resistant
B_{s-1}	Fil⁺Her⁻Exr⁻	B	Hill	Sensitive
26x	Fil⁻Her⁻Exr⁻Thr	B_{s-1}	Witkin	Sensitive
26xA3	Fil⁻Her⁻Exr⁺⁺Thr⁻Pro	26xA2*	Witkin	Resistant

* 26xA2 (Fil⁻Her⁻Exr⁺) derived from 26x.

The convention for the different phenotypes is given below.

Phenotype	Characteristic
Fil	Filament formation, etc.
Her	Host cell reactivation and excision repair capacity.
Exr	Sensitivity to X-ray damage.

Growth of organisms

(i) *Resting phase*. The bacteria were grown in a tryptone medium (Cox, 1966a) at 37° C. with shaking. After 18 hr. growth the bacteria were harvested by centrifugation and were resuspended in double-glass-distilled water. This suspension was used to generate the aerosols.

(ii) *Log phase*. The bacteria were grown in nutrient broth, as for the resting phase bacteria. After 18 hr., 0·1 ml. of this culture was used to inoculate a fresh batch of nutrient broth (100 ml.). After 4 hr. growth at 37° C., with shaking, the bacteria were harvested by centrifugation and resuspended in fresh nutrient broth. This culture was used to generate the aerosols.

Aerosol apparatus

A rotating drum apparatus was used, as described by Cox (1966*a*). Aerosol samples were collected by impingers, as described by Cox (1966*a*). Usually the experimental conditions were 20% RH and 26·8° C.

Assay

Suspension and aerosol samples were diluted as required and plated on nutrient agar. After overnight growth at 37° C., the colonies were counted.

Tracer

Bacillus subtilis var. *niger* spores were used as a tracer (Cox, 1966*a*; Anderson & Cox, 1967). The ratio of *Escherichia coli* to spores was calculated for the suspension and for the aerosol samples. Viabilities were taken as the ratio of coli to spore count, normalized to the ratio in the spray suspension as being 100% survival. The latter was measured before and after spraying.

Net DNA synthesis

For these experiments the strains of *Escherichia coli* were grown in a lactate medium composed of 0·004 g. P as orthophosphate, 10 g. sodium lactate, 5·2 g. $NaCl$, 1·8 g. K_2SO_4, 0·12 g. $MgSO_4$, 1·0 g. NH_4Cl, 0·5 g. Bactopeptone, per 1000 ml. distilled water. The pH was 6·8. The suspension used to produce aerosols was then prepared as for resting phase cultures, except that the spores were omitted.

Since larger populations than are normally collected from aerosols were required for the determination of net DNA synthesis, suspensions containing 10^{11} bacteria/ml. were disseminated with a 3-jet Collison spray and aerosol samples (30 min. aerosol age) were collected for 2·5 min. in an impinger. The optical density of the collected aerosol (determined by a Beckman spectrophotometer) was matched by that of a control, obtained by diluting the suspension used for generating the aerosol. In all cases the bacteria in the aerosol sample and in the control were suspended in the lactate medium plus deoxyadenosine (250 μg/ml.) at a density of 1·1 to 1·7 × 10^8 bacteria/ml. The two suspensions were rapidly warmed to 37° C. and at t = 0, 0·3 ml. [^{3}H]thymidine was added to each suspension at 37° C.: the suspensions then contained 38 μg/ml. and 1·2 μCi/ml. of [^{3}H]thymidine. At intervals (the shortest being 30 sec.) 0·2 ml. was removed from each suspension and pipetted onto separate Millipore filters (25 mm., 0·22 μm.) previously wetted with ice-cold 1 N-NaOH. After filtration the bacteria were washed with a total of 15 ml. of ice-cold 5% (w/v) trichloroacetic acid (TCA), and then with absolute ethanol. After drying, the bacterial samples on the Millipore filters were put into separate 10 ml. volumes of scintillation fluid ('Permablend' from Packard Instruments). The amount of [^{3}H]thymidine incorporated into the cold TCA insoluble material was determined in a Packard Scintillation Spectrometer (Model 4322).

Oxygen uptake

The bacteria were grown, aerosolized and collected, as for the experiments to measure net DNA synthesis, except that the collecting fluid was phosphate buffer (Cox, 1966a). A suspension containing 2×10^8 bacteria/ml. was put in the side-arm of a Warburg flask, KOH was in the centre well, and the lactate medium was in the main compartment of the flask. The metabolic activity of bacteria collected from the aerosol, and of an unstressed control, was determined by the Warburg technique, at 37° C.

All survival, net DNA synthesis and metabolic activity experiments were performed between two and five times.

RESULTS

Aerosol survival of Escherichia coli *B/r*

Fig. 1 shows the aerosol survival of *Escherichia coli* B/r (Hill) at 20% RH (26·8° C.) for log and resting phase bacteria in air and nitrogen (> 99·997 %)
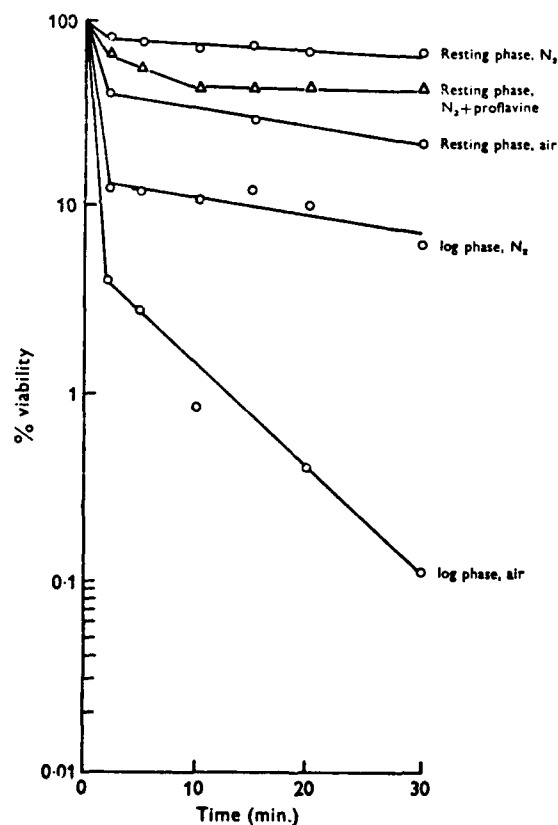

Fig. 1. Aerosol survival of *Escherichia coli* B/r (Hill) at 20 % relative humidity and 26·8° C

(Matheson Company). Also included are results obtained when the collected aerosol samples were plated onto nutrient agar plus proflavine (5 μg./ml.) (an excision repair inhibitor, Witkin, 1963; Lieb, 1964). The results show that survival of resting phase and of log phase bacteria was greater in nitrogen than in air. Under comparable conditions, resting phase bacteria were more aerosol stable than were log phase bacteria. The addition of proflavine to the nutrient agar had only a slight inhibitory effect.
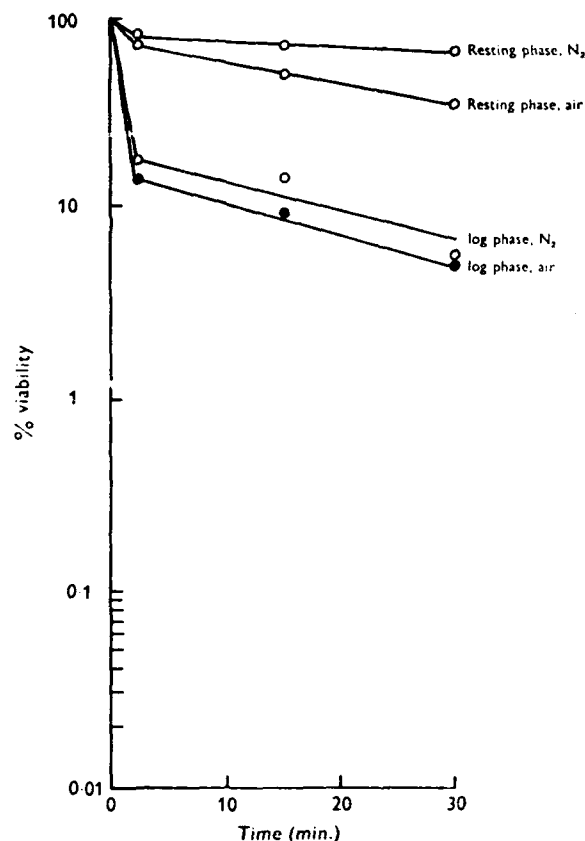

Fig. 2. Aerosol survival of *Escherichia coli* B/r (Witkin) at 20% relative humidity and 26·8 C.

Fig. 2 is similar to Fig. 1 except that the Witkin strain of *Escherichia coli* B/r was used. The results suggest that the Witkin strain is a little more resistant to aerosol damage than is the strain of Hill, especially for log phase bacteria in air. Again only a slight inhibitory effect of the addition of proflavine (5 μ./ml.) to the nutrient agar was found, as for Fig. 1.

Aerosol survival of Escherichia coli B_{s-1}

The data of Fig. 3 were obtained under the same conditions as for Figs. 1 and 2, but using the B_{s-1} strain. As with the B/r strains, survival in air was less than in

nitrogen, although with strain B_{s-1} the difference was much greater. Log phase bacteria were again more unstable than resting phase bacteria, for comparable conditions.
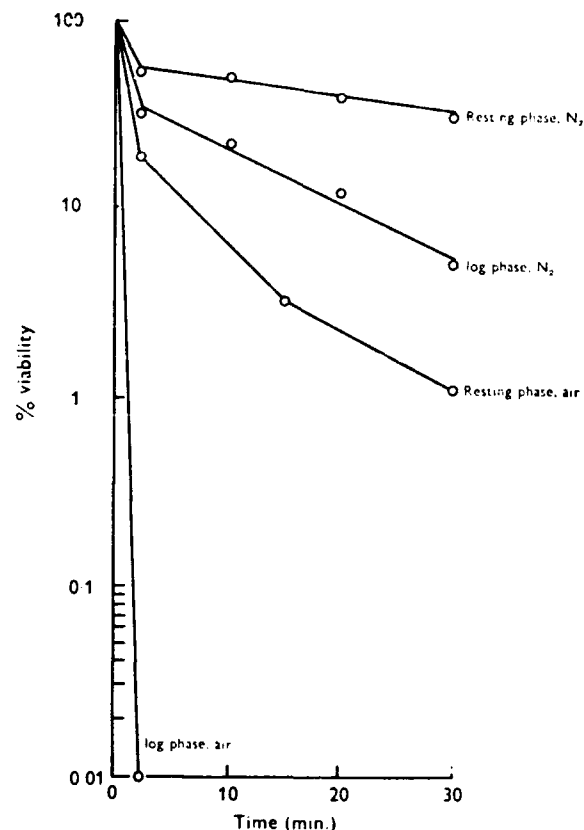

Fig. 3. Aerosol survival of *Escherichia coli* B_{s-1} at 20 % relative humidity and 26·8° C.

Aerosol survival of Escherichia coli 26x

As with the other strains, air is toxic compared with nitrogen (Fig. 4). The actual survival under the different conditions was similar to that of strain B/r (Figs. 1 and 2).

Aerosol survival of Escherichia coli 26xA3

As with *Escherichia coli* 26x (Fig. 4) the results (Fig. 5) for *E. coli* 26xA3 are similar to those of strain B/r (Figs. 1 and 2), except for resting phase bacteria in air, which resembled strain B_{s-1} behaviour.

Net DNA synthesis in aerosol-stressed Escherichia coli *B/r*

Net DNA synthesis in aerosol-stressed *Escherichia coli* B/r was less than that in the unstressed bacteria (Fig. 6). However, the inhibition was the same for

samples collected from aerosols stored in nitrogen and air, i.e. the presence of oxygen did not have any effect on net DNA synthesis of stressed *E. coli* B/r. The 30 min. viabilities at 36 % RH and 26·8° C., were 24 % in nitrogen and 2·1 % in air.
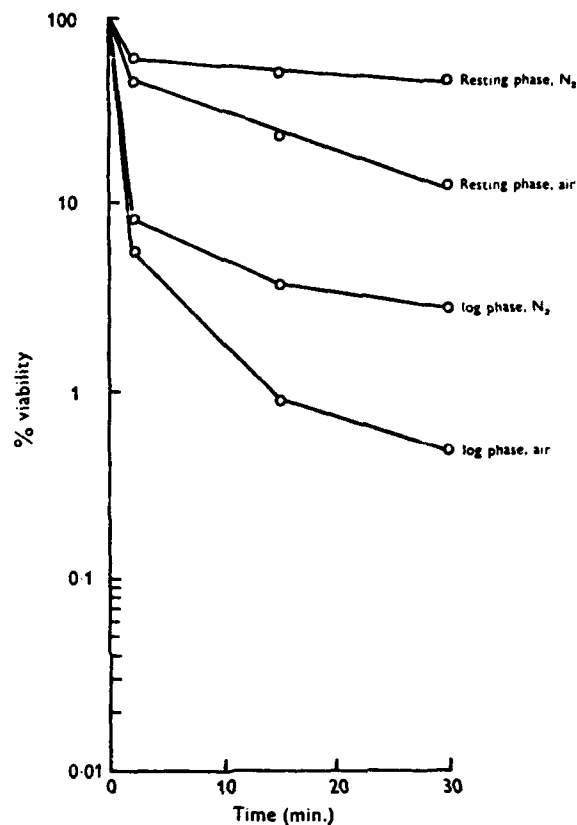

Fig. 4. Aerosol survival of *Escherichia coli* 26x at 20 % relative humidity and 26·8° C.

Net DNA synthesis in aerosol-stressed Escherichia coli B_{s-1}

The ability of aerosol-stressed *Escherichia coli* B_{s-1} to synthesize DNA was inhibited compared with unstressed controls (Fig. 7). The result obtained was similar to that with *E. coli* B/r (Fig. 6) even though the 30 min. viabilities at 36 % RH and 26·8° C. of strain B_{s-1} were 13 % in nitrogen and 0·014 % in air. Again, oxygen was not involved in causing inhibition of net DNA synthesis (Fig. 7).

The kinetics of [³H]thymidine incorporation in *Escherichia coli* B/r (Fig. 6) and *E. coli* B_{s-1} (Fig. 7) were not exactly the same, especially at 30 sec.; the reason for this difference is not known, but is not an artifact resulting from insufficient washing.
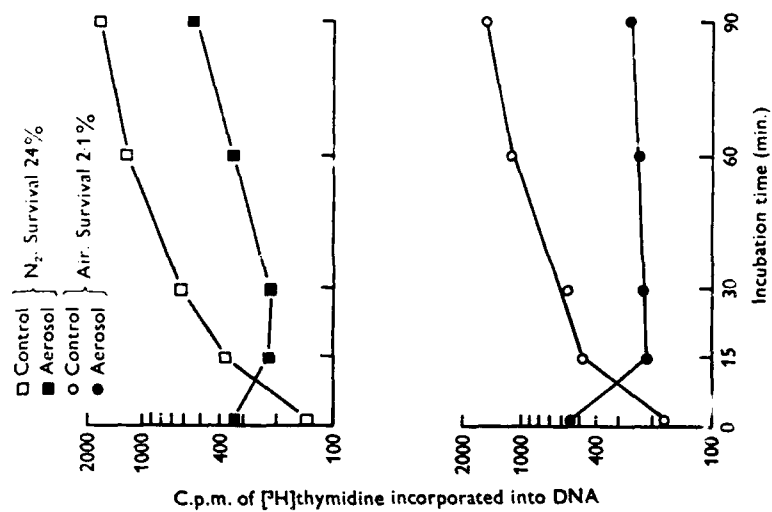
Fig. 6. Net DNA synthesis in *Escherichia coli* B/r (Hill) collected from an aerosol stored at 36% relative humidity and 26·8° C. 30 min. survival in nitrogen 24% and in air 2·1%.
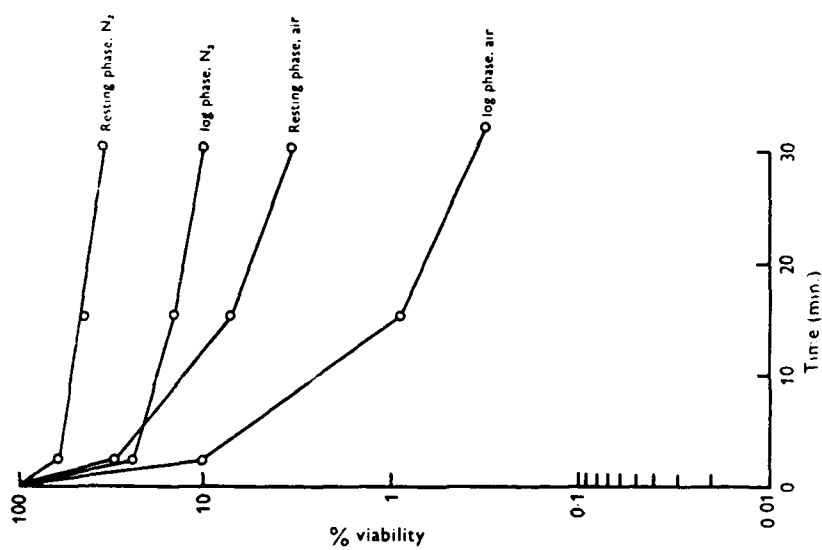

Fig. 5. Aerosol survival of *Escherichia coli* 26xA3 at 20% relative humidity and 26·8° C.

Oxygen uptake by aerosol-stressed Escherichia coli

The data of Figs. 8 and 9 show that aerosol-stressed *Escherichia coli* B/r and B_{s-1} have lower metabolic activity than unstressed control bacteria. The degree of reduction in metabolic activity was independent of the presence of oxygen in the aerosol phase.
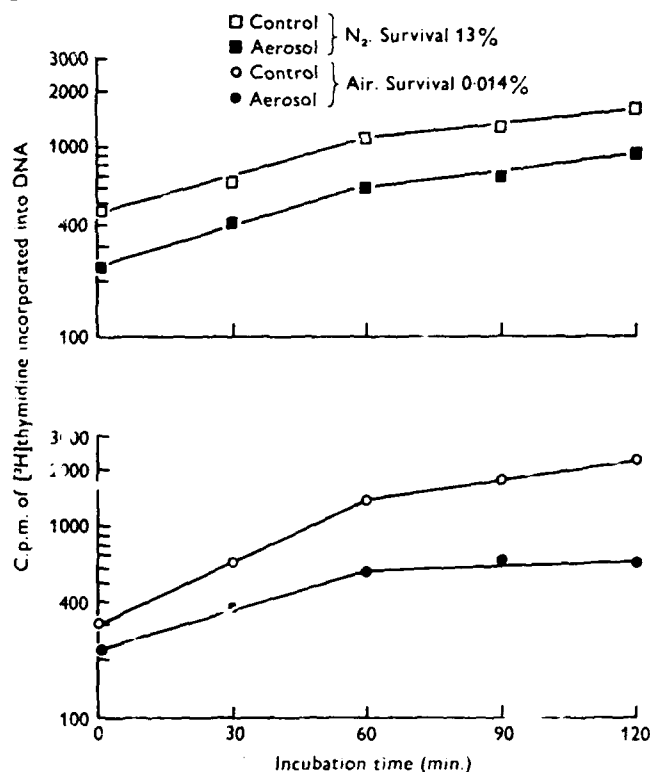

Fig. 7. Net DNA synthesis in *Escherichia coli* B_{s-1} collected from an aerosol stored at 36% relative humidity and 26·8° C. 30 min. survival in nitrogen 13% and in air 0·014%.

DISCUSSION

Aerosol survival data are summarized in Table 1, which gives the 30 min. survival values of the different strains (resting and log phase) in air and nitrogen, at 20% RH and 26·8° C.

As seen from Table 1 and Figs. 1-5, there does not appear to be an overall correlation between aerosol survival in air, or in nitrogen, and the Exr. Exr phenotypes of the six strains of *Escherichia coli*. For log phase bacteria in air there may be a correlation with Fil, since *E. coli* B and B_{s-1} are both Fil^+ and in air both are much less stable than the Fil^- strains, i.e. Fil^- for these conditions could be involved in oxygen-induced damage. However, preliminary data show log phase *E. coli* B to be more unstable in nitrogen than is *E. coli* B_s (Table 1).

41-2

When decay in air is corrected for the decay in nitrogen, the toxic action of oxygen is much greater for $E.\ coli$ B_{s-1} than for $E.\ coli$ B, i.e. the apparent correlation with Fil+ may be an artifact. Further work is required to substantiate the possible rôle of Fil.
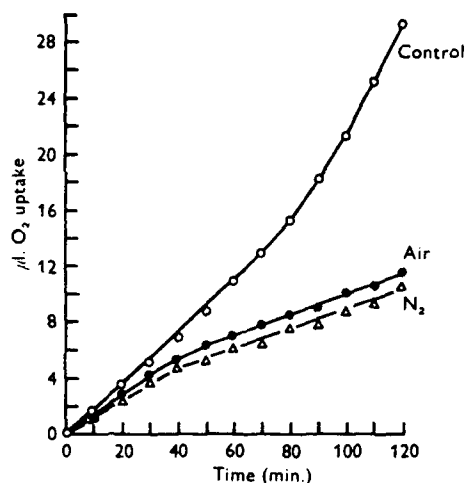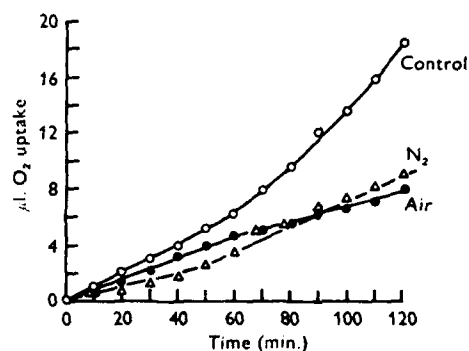

Fig. 8

Fig. 9

Fig. 8. Oxygen uptake by *Escherichia coli* B/r collected from aerosols in air (●) and in nitrogen (△). (○) Control.

Fig. 9. Oxygen uptake by *Escherichia coli* B_{s-1} collected from aerosols in air (●) and in nitrogen (△). (○) Control.

Table 1. *Survival values* (30 min.) *of strains of* Escherichia coli *stored at* 20% *relative humidity* (26·8° C.)

		Survival % log phase		Survival % resting phase	
Strain	Phenotype	Air	Nitrogen	Air	Nitrogen
B/r (Hill)	Fil−Her−Exr+	0·1	7	21	75
B/r (Witkin)	Fil−Her+Exr+	5	7	35	65
B*	Fil−Her+Exr+	0†	0·3	1	76
B_{s-1}	Fil+Her−Exr−	0‡	5	1	30
26x	Fil−Her−Exr−	0·5	3	12	44
26xA3	Fil−Her+Exr+	0·3	10	3	35

* Cox, unpublished preliminary data.
† 2 min. value 1·2%.
‡ 2 min. value 0·01%.

In air, survival was always lower than in nitrogen, although the degree of difference depended upon the strain of *Escherichia coli*. These results may be explained in terms of a toxic action of oxygen (Ferry, Brown & Damon, 1958; Hess, 1965; Cox, 1966a, 1968b, 1970, 1971; Cox & Baldwin, 1966, 1967; Cox &

Heckly, 1972; Benbough, 1967, 1969; Webb, 1967, 1969). Even though the presence of oxygen decreased survival, this decrease was not due to the influence of oxygen on net DNA synthesis or metabolic activity (Figs. 6, 7, 8 and 9; Benbough, 1967).

In nitrogen, strains of *Escherichia coli* are not completely stable, i.e. there is an oxygen-independent death mechanism (Figs. 1–5, Table 1; Cox, 1966a, 1968a, b, 1970, 1971; Cox & Baldwin, 1966, 1967; Benbough, 1967, 1969; Webb, 1967, 1969). This might be explained in terms of only partial repair to aerosol-induced DNA damage. However, the DNA repair mechanisms for radiation-induced DNA damage do not appear to operate for aerosol damage, because repair capability of radiation-induced DNA damage and aerosol survival are not related in nitrogen atmospheres (Figs. 1–5, Table 1). Also, the presence of proflavine (5 μg/ml.) in the plating medium did not greatly decrease the survival of two strains of *E. coli* B/r, even though proflavine inhibits host cell reactivation (Witkin, 1963; Lieb, 1964), i.e. excision repair. However, Webb (1969) reported a marked inhibition of *E. coli* B/r growth caused by the addition of proflavine (5 μg/ml.) to nutrient agar. The cause of this discrepancy is not known. Net DNA incorporation of [^{3}H]thymidine is partially inhibited in *Escherichia coli* B/r and *E. coli* B_{s-1} stored in air and nitrogen at 36 % RH (Figs. 6 and 7). This reflects a partial inactivation of DNA synthesis, or an increased rate of DNA breakdown, which is independent of the presence of oxygen in the aerosol phase. The reduced metabolic activity of *E. coli* B/r and B_{s-1} shown in Figs. 8 and 9 is also independent of the presence of oxygen in the aerosol phase. Reduced metabolic activity could account for the reduced net DNA synthesis in these organisms. A determination of the physical integrity of DNA in bacteria collected from the aerosol might elucidate this question.

The data presented in this paper indicate that mechanisms involved in repair of radiation-induced damage (viz. Fil, Hcr, Exr phenotypes) do not operate for aerosol-induced damage. However, it is possible that other unknown mechanisms repair aerosol-induced dehydration-rehydration damage.

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